

Saidha  
09/812502  
Seq. ID 16

09/812502

L1 FILE 'REGISTRY' ENTERED AT 13:05:45 ON 18 DEC 2002  
4 S CP..EEKNDRICTNCCAG.KG/SQSP  
L1 ANSWER 1 OF 4 REGISTRY COPYRIGHT 2002 ACS  
RN 447480-29-7 REGISTRY  
CN 57: PN: W002063011 SEQID: 57 unclaimed protein (9CI) (CA INDEX  
NAME)  
CI MAN  
SQL 368

SEQ 1 KACTLNCDPR IAYGVCPRSE EKKNDRICTN CCAGTKGCKY FSDDGTFVCE  
=====   
51 GESDPRNPKA CTLNCDPRIA YGVCPRSEK KNDRICTNCC AGTKGCKYFS  
=====   
101 DDGTFVCEGE SDPRNPKACP RNCDPRIAYG ICPLAEEKKN DRICTNCCAG  
=====   
151 KKGCKYFSDD GTFVCEGESD PKNPKACPRN CDGRIAYGIC PLSEEKKNDR  
=====   
201 ICTNCCAGKK GCKYFSDDGT FVCEGESDPK NPKACPRNCD GRIAYGICPL  
=====   
251 SEEKNDRICT TNCCAGKKGC KYFSDDGTFV CEGESDPRNP KACPRNCDGR  
=====   
301 IAYGICPLSE EKKNDRICTN CCAGKKGCKY FSDDGTFICE GESEYASKVD  
=====   
351 EYVGEVENDL QKSKVAVS

HITS AT: 16-37, 74-95, 132-153, 190-211, 248-269, 306-327

\*\*RELATED SEQUENCES AVAILABLE WITH SEQLINK\*\*

REFERENCE 1: 137:165270

L1 ANSWER 2 OF 4 REGISTRY COPYRIGHT 2002 ACS  
RN 280151-61-3 REGISTRY  
CN Proteinase inhibitor, potato, II (Nicotiana alata clone Na-PIIV  
four-domain isoform precursor) (9CI) (CA INDEX NAME)  
OTHER NAMES:  
CN GenBank AF105340-derived protein GI 6492109  
CN Potato proteinase inhibitor II (Nicotiana alata isoform PI IV  
precursor)  
CI MAN  
SQL 281

SEQ 1 MAAHRVSFLA LLLLFGMSLL VSNVEHADAK ACTLNCDPRI AYGVCPRSEE  
=====   
51 KKNDRICTNC CAGTKGCKYF SDDGTFVCEG ESDPRNPKAC TLNCDPRIAY  
=====   
101 GVCPRSEEEK NDRICTNCCA GTKGCKYFSD DGTFVCEGES DPKNPKACPR  
=====   
151 NCDPRIAYGI CPLSEEKND RICTNCCAGK KGCKYFSDDG TfvCEGESDP  
=====   
201 RNPKACPRNC DGRIAYGICP LSEEKKNDR CTNCCAGKKG CKYFSDDGTF  
=====   
251 ICEGESEYAS KVDEYVGEVE NDLQKSKVAV S

HITS AT: 45-66, 103-124, 161-182, 219-240

REFERENCE 1: 133:85962

L1 ANSWER 3 OF 4 REGISTRY COPYRIGHT 2002 ACS

Searcher : Shears 308-4994

09/812502

RN 157858-12-3 REGISTRY  
CN Proteinase inhibitor (Nicotiana alata clone Na-PI-II reduced) (9CI)  
(CA INDEX NAME)  
CI MAN  
SQL 368

SEQ 1 KACTLNCDPR IAYGVCPRSE EKKNDRICTN CCAGTKGCKY FSDDGTFVCE  
=====   
51 GESDPRNPKA CTLNCDPRIA YGVCPRSEK KNDRICTNCC AGTKGCKYFS  
=====   
101 DDGTFVCEGE SDPRNPKACP RNCDPRIAYG ICPLAEEKKN DRICTNCCAG  
=====   
151 KKGCKYFSDD GTFVCEGESD PKNPKACPRN CDGRIAYGIC PLSEEKKNDR  
=====   
201 ICTNCCAGKK GCKYFSDDGT FVCEGESDPK NPKACPRNCD GRIAYGICPL  
=====   
251 SEEKKNDRIC TNCCAGKKGC KYFSDDGTFV CEGESDPRNP KACPRNCDGR  
=====   
301 IAYGICPLSE EKKNDRICTN CCAGKKGCKY FSDDGTFICE GESEYASKVD  
=====   
351 EYVGEVENDL QKSKVAVS

HITS AT: 16-37, 74-95, 132-153, 190-211, 248-269, 306-327

\*\*RELATED SEQUENCES AVAILABLE WITH SEQLINK\*\*

REFERENCE 1: 121:199522

L1 ANSWER 4 OF 4 REGISTRY COPYRIGHT 2002 ACS  
RN 148499-83-6 REGISTRY  
CN Proteinase inhibitor (Nicotiana alata clone Na-PI-II precursor  
reduced) (9CI) (CA INDEX NAME)  
CI MAN  
SQL 397

SEQ 1 MAVHRVSFLA LLLLFGMSLL VSNVEHADAK ACTLNCDPRI AYGVCPRSEE  
=====   
51 KKNDRICTNC CAGTKGCKYF SDDGTFVCEG ESDPRNPKAC TLNCDPRIAY  
=====   
101 GVCPRSEEEK NDRICTNCCA GTKGCKYFSD DGTFVCEGES DPRNPKACPR  
=====   
151 NCDPRIAYGI CPLAEEKKND RICTNCCAGK KGCKYFSDDG TFVCEGESDP  
=====   
201 KNPACPRNC DGRIAYGICP LSEEKKNDR CTNCCAGKKG CKYFSDDGTF  
=====   
251 VCEGESDPKN PKACPRNCDG RIAYGICPLS EEKKNDRIC NCCAGKKGCK  
=====   
301 YFSDDGTFVC EGESDPRNPK ACPRNCDGRI AYGICPLSEE KKNDRICTNC  
=====   
351 CAGKKGCKYF SDDGTFICEG ESEYASKVDE YVGEVENDLQ KSKVAVS  
=====

HITS AT: 45-66, 103-124, 161-182, 219-240, 277-298, 335-356

REFERENCE 1: 123:279564

REFERENCE 2: 119:45231

FILE 'HCAPLUS' ENTERED AT 13:06:56 ON 18 DEC 2002  
L2 5 S L1

Searcher : Shears 308-4994

09/812502

L2 ANSWER 1 OF 5 HCAPLUS COPYRIGHT 2002 ACS  
ACCESSION NUMBER: 2002:615859 HCAPLUS  
DOCUMENT NUMBER: 137:165270  
TITLE: Sequence homologs of floral defensin-like  
proteins and their use in improving plant  
disease resistance  
INVENTOR(S): Anderson, Marilyn Anne; Lay, Fung Tso; Heath,  
Robyn Louise  
PATENT ASSIGNEE(S): Hexima Ltd., Australia  
SOURCE: PCT Int. Appl., 164 pp.  
CODEN: PIXXD2  
DOCUMENT TYPE: Patent  
LANGUAGE: English  
FAMILY ACC. NUM. COUNT: 1  
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2002063011	A1	20020815	WO 2002-AU123	20020208
W:	AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, OM, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM			
RW:	GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG			

PRIORITY APPLN. INFO.: US 2001-267271P P 20010208

OTHER SOURCE(S): MARPAT 137:165270

AB CDNAs encoding plant flower defensin (.gamma.-thionin)-like proteins are identified for use in improving plant resistance to pests including insects, microorganisms, fungi and/or viruses. The plants may be monocotyledonous or dicotyledonous plants and are in particular, crop plants and ornamental flowering plants. The cloned genes may be used to manuf. the protein for agrochem. applications. The floral defensin-like mols. or genes encoding them may be used alone or in combination with other agents such as a proteinase inhibitor precursor or a nucleic acid mol. encoding same or other mols. or their encoding nucleotide sequences. The gene was identified in flowers of Nicotiana glauca after PCR with primers derived from .gamma.-thionins. Tissue distribution of the mRNA and the protein is consistent with a defensive protein for flowers. The protein shows the sequence motifs typical of a defensin. The protein was effective in inhibiting the growth of a no. of plant pathogenic fungi. Transgenic Nicotiana glauca expressing the gene from the 35S promoter gave rise to plants that slowed the growth of Helicoverpa punctigera fed on them.

IT 447480-29-7

RL: PRP (Properties)

(unclaimed protein sequence; sequence homologs of floral defensin-like proteins and their use in improving plant disease resistance)

REFERENCE COUNT: 3 THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN

Searcher : Shears 308-4994

09/812502

THE RE FORMAT

L2 ANSWER 2 OF 5 HCAPLUS COPYRIGHT 2002 ACS  
ACCESSION NUMBER: 2000:286780 HCAPLUS  
DOCUMENT NUMBER: 133:85962  
TITLE: Identification of a novel four-domain member of  
the proteinase inhibitor II family from the  
stigmas of *Nicotiana glauca*  
AUTHOR(S): Miller, Elizabeth A.; Lee, Marcus C. S.;  
Atkinson, Angela H. O.; Anderson, Marilyn A.  
CORPORATE SOURCE: Department of Biochemistry and Genetics, LaTrobe  
University, Bundoora, 3083, Australia  
SOURCE: Plant Molecular Biology (2000), 42(2), 329-333  
CODEN: PMBIDB; ISSN: 0167-4412  
PUBLISHER: Kluwer Academic Publishers  
DOCUMENT TYPE: Journal  
LANGUAGE: English

AB Proteinase inhibitors (PIs) of the potato type II family have been  
identified in a no. of solanaceous species. Most family members  
have two PI domains which are specific for either chymotrypsin or  
trypsin. More recently family members have been described with  
three or six repeated PI domains. Here we describe a novel  
four-domain family member produced in the stigmas and leaves of the  
ornamental tobacco, *Nicotiana glauca*, which has high sequence  
identity with a six-domain member from the same species. Both  
proteins are produced as precursors that enter the secretory pathway  
and are subsequently processed into a series of 6 kDa PIs. The  
four- and six-domain precursor proteins were isolated from immature  
stigmas and characterized by mass spectrometry which revealed that  
both proteins had been trimmed at the N-terminus, at a position  
corresponding to the predicted signal peptide cleavage site.  
Furthermore, no post-translational modifications were apparent.

IT 280151-61-3

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL  
(Biological study)  
(amino acid sequence; identification, cloning and sequence of  
novel four-domain member of potato proteinase inhibitor II family  
from stigmas of *Nicotiana glauca*)

REFERENCE COUNT: 19 THERE ARE 19 CITED REFERENCES AVAILABLE  
FOR THIS RECORD. ALL CITATIONS AVAILABLE  
IN THE RE FORMAT

L2 ANSWER 3 OF 5 HCAPLUS COPYRIGHT 2002 ACS  
ACCESSION NUMBER: 1995:592660 HCAPLUS  
DOCUMENT NUMBER: 123:279564  
TITLE: Characterization of the protease processing  
sites in a multidomain proteinase inhibitor  
precursor from *Nicotiana glauca*  
AUTHOR(S): Heath, Robyn L.; Barton, Peter A.; Simpson,  
Richard J.; Reid, Gavin E.; Lim, Guan; Anderson,  
Marilyn A.  
CORPORATE SOURCE: Plant Cell Biology Research Centre, School of  
Botany, University of Melbourne, Victoria,  
Australia  
SOURCE: European Journal of Biochemistry (1995), 230(1),  
250-7  
CODEN: EJBCAI; ISSN: 0014-2956  
PUBLISHER: Springer

Searcher : Shears 308-4994

09/812502

DOCUMENT TYPE: Journal  
LANGUAGE: English

AB A gene encoding a 40.3-kDa serine proteinase inhibitor (PI) precursor is expressed at high levels in the stigma of the ornamental tobacco, *Nicotiana glauca*. The precursor is processed proteolytically in vivo to release five homologous proteinase inhibitors of approx. 6 kDa, as well as two flanking peptides. The five PIs have been purified from stigmas and identified by N-terminal sequencing, electrospray mass spectrometry and inhibition activity against chymotrypsin or trypsin. One of the PIs inhibits chymotrypsin and the other four are most active on trypsin. Cleavage occurs in a linker region (EEKKND) that is repeated six times in the precursor mol. In the plant, the initial cleavage probably occurs between asparagine and the aspartate residues and ragged ends are formed by subsequent trimming. In vitro, the protease-sensitive linker region is selectively cleaved by the endoproteinases Asp-N, Glu-C and Lys-C to release fully active approx. 6-kDa PIs that are resistant to further proteolytic digestion. The precursor, produced by a recombinant baculovirus, inhibits chymotrypsin more effectively than trypsin. The stoichiometry of 2.6 trypsin mols./1 precursor mol. indicates that processing is required to activate or expose all of the four trypsin inhibitory sites.

IT **148499-83-6**, Proteinase inhibitor (*Nicotiana glauca* clone Na-PI-II precursor reduced)  
RL: PRP (Properties)  
(protease processing sites in multidomain proteinase inhibitor precursor from *Nicotiana glauca*)

L2 ANSWER 4 OF 5 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1994:599522 HCAPLUS

DOCUMENT NUMBER: 121:199522

TITLE: A plant type II serine proteinase inhibitor and the gene encoding it

INVENTOR(S): Anderson, Marilyn Anne; Atkinson, Angela Hilary; Heath, Robyn Louise; Clarke, Adrienne Elizabeth

PATENT ASSIGNEE(S): University of Melbourne, Australia

SOURCE: PCT Int. Appl., 82 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent

LANGUAGE: English

FAMILY ACC. NUM. COUNT: 2

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9413810	A1	19940623	WO 1993-AU659	19931216
W:	AT, AU, BB, BG, BR, BY, CA, CH, CZ, DE, DK, ES, FI, GB, HU, JP, KP, KR, KZ, LK, LU, LV, MG, MN, MW, NL, NO, NZ, PL, PT, RO, RU, SD, SE, SK, UA, US, UZ, VN			
RW:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG			
CA 2151933	AA	19940623	CA 1993-2151933	19931216
AU 9456891	A1	19940704	AU 1994-56891	19931216
AU 680855	B2	19970814		
EP 674712	A1	19951004	EP 1994-902551	19931216
R:	AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, PT, SE			

Searcher : Shears 308-4994

09/812502

JP 11346788	A2	19991221	JP 1999-128615	19931216
JP 2000175581	A2	20000627	JP 2000-3716	19931216
US 6031087	A	20000229	US 1995-454295	19950901
US 6261821	B1	20010717	US 1999-431500	19991101
US 6440727	B1	20020827	US 1999-431498	19991101
US 6451573	B1	20020917	US 1999-431499	19991101

PRIORITY APPLN. INFO.:

AU 1992-6399	A	19921216
JP 1994-513583	A3	19931216
WO 1993-AU659	W	19931216
US 1995-454295	A3	19950901

AB A type II serine proteinase inhibitor that may be of use in plant resistance to insect pests is identified and the gene encoding it is cloned and expressed for use in the development of insect-resistant transgenic plants. The protein is manufd. as a heterooligomer of trypsin and chymotrypsin-specific subunits. A cDNA for the protein was cloned from a stigma and style cDNA bank of *Nicotiana glauca* by screening with probes derived from the potato and tomato inhibitors. The protein encoded by the cDNA is made up of six domains that are imperfect repeats, two of these are chymotrypsin-inhibiting domains and four are trypsin-inhibiting domains. Expression of the gene was detectable in stigma and style and was induced in leaf upon wounding. The cDNA for this precursor form was expressed in Sf9 cells. The inhibitor was active against the gut proteinases of a no. of insect pests.

IT 157858-12-3

RL: BIOL (Biological study)

(amino acid sequence of and cloning and expression of cDNA for)

L2 ANSWER 5 OF 5 HCAPLUS COPYRIGHT 2002 ACS

ACCESSION NUMBER: 1993:445231 HCAPLUS

DOCUMENT NUMBER: 119:45231

TITLE: Proteinase inhibitors in *Nicotiana glauca* stigmas are derived from a precursor protein which is processed into five homologous inhibitors

AUTHOR(S): Atkinson, Angela H.; Heath, Robyn L.; Simpson, Richard J.; Clarke, Adrienne E.; Anderson, Marilyn A.

CORPORATE SOURCE: Sch. Bot., Univ. Melbourne, Parkville, 3052, Australia

SOURCE: Plant Cell (1993), 5(2), 203-13  
CODEN: PLCEEW; ISSN: 1040-4651

DOCUMENT TYPE: Journal

LANGUAGE: English

AB A cDNA clone, NA-PI-II, encoding a protein with partial identity to proteinase inhibitor (PI) II of potato and tomato has been isolated from a cDNA library constructed from *Nicotiana glauca* stigma and style mRNA. The cDNA encodes a polypeptide of 397 amino acids with a putative signal peptide of 29 amino acids and six repeated domains, each with a potential reactive site. Domains 1 and 2 have chymotrypsin-specific sites and domains 3, 4, 5, and 6 have sites specific for trypsin. In situ hybridization expts. demonstrated that expression of the gene is restricted to the stigma of both immature and mature pistils. Peptides with inhibitory activity toward chymotrypsin and trypsin have been isolated from stigmas of *N. glauca*. The N-terminal amino acid sequence obtained from this protein prepn. corresponds to six regions in the cDNA clone NA-PI-II. The purified PI protein prepn. is likely to be composed of a mixt. of up to five similar peptides of .apprx.6 kD, produced

09/812502

in vivo by proteolytic processing of a 42-kD precursor. The PI may function to protect the reproductive tissue against potential pathogens.

IT 148499-83-6, Proteinase inhibitor (Nicotiana alata clone Na-PI-II precursor)

RL: BIOL (Biological study)

(amino acid sequence and proteolytic processing in stigma of)

FILE 'HOME' ENTERED AT 13:07:51 ON 18 DEC 2002